Effects of H2A Histone Variants on DNA Sequence and Nucleosome Structure using Coarse Grain Simulations

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nucleic acid-peptide binding was mostly due to interactions between the peptide and phosphate backbone of nucleic acids, providing an explanation for the lack of sequence specificity observed experimentally. These insights regarding nucleic acid binding of buforin II and DesHDAP1, paired with a deeper understanding of the peptides' structures and membrane interactions, are necessary for development of novel pharmaceutical applications using AMPs.

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Oxygen-to-Sulfur Substitution of DNA Phosphate Entropically Enhances Protein-DNA Affinity

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For some proteins, dithioation of DNA phosphate is known to enhance binding affinities. For the Antennapedia (Antp) homedomain - DNA complex, we have mechanistically characterized this phenomenon by integrated use of fluorescence, isothermal calorimetry (ITC), NMR spectroscopy, and X-ray crystallography. Through ITC and fluorescence, we found that this affinity enhancement is entropy-driven. By NMR, we investigated the ionic hydrogen bonds and internal motions of lysine side-chain $NH3^+$ groups involved in ion pairs with DNA. By X-ray crystallography, we compared the structures of complexes with and without dithioation of the phosphate. Our NMR and X-ray data demonstrate that the lysine side chain in contact with the DNA phosphate becomes more dynamic upon dithioation. Our thermodynamic, structural, and dynamic investigations collectively show that the affinity enhancement by the oxygen-to-sulfur substitution in DNA phosphate is largely due to an entropic gain arising from mobilization of the intermolecular ion pair at the protein-DNA interface.

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Role of the Moiety Chirality in Determining the DNA Binding Characteristics of Threading Intercalators

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Threading intercalators are small molecules that bind to DNA by threading their ancillary groups through the DNA bases to intercalate their middle planar section between the DNA base pairs. The high binding affinity and slow dissociation rates of threading intercalators have put them in the class of prospective anti-cancer drugs. In this study we explore the binding of a specific threading intercalator, the binuclear ruthenium complex ΛΛ-P (ΛΛ-[μ-bidppz(phen)$_4$Ru$_2$]$^{4+}$) using optical tweezers. A single DNA molecule is held at a constant force and ΛΛ-P is introduced to the system in varying concentrations until equilibrium is achieved. Measurements of DNA extension at various concentrations of ΛΛ-P as a function of time provide the DNA equilibrium binding affinity and binding kinetics for this molecule. Preliminary data analysis at constant force suggests that ΛΛ-P exhibits significantly faster binding kinetics compared to the very similar ΔΔ-P [μ-bidppz(phen)$_4$Ru$_2$]$^{4+}$. These complexes have the same chemical structure and only differ in their chirality, which suggests that the left handed (ΛΛ) threading moieties require less DNA structural distortion for threading compared with the right handed (ΔΔ) threading moieties.

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Direct Observation of the Stepping Behavior of E. Coli UvrD Helicase

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Helicases are a diverse set of enzymes that play an essential role in genome maintenance by converting chemical energy into the mechanical work of unzipping nucleic acid duplexes. E. coli UvrD is a prototypical superfamily 1 helicase involved in methyl-directed mismatch repair and nucleotide excision repair. Recent measurements have shown that it is able to both unwind and rezip the DNA duplex. Structural, ensemble kinetic, and single-molecule studies have provided insights into the unwinding mechanism of this helicase, but there has not yet been a direct observation of the individual motor steps involved in the process. Here, we use high-resolution optical tweezers to observe directly the stepping behavior of UvrD at a single-molecule level as it unwinds and rezips a DNA hairpin. Interestingly, we measure a step size of 3-4 base pairs for both unwinding and rezipping activities. Furthermore, our analysis of the stepping kinetics indicates that a single rate-limiting step governs the process, which is consistent with ensemble studies measuring an unwinding step size of 4-5 base pairs per rate-limiting step. However, previous studies have determined that 1 ATP molecule is hydrolyzed per base pair translocated, and would predict 3-4 rate-limiting steps per unwinding step. Our results therefore suggest a mechanism whereby UvrD rapidly unwinds 3-4 base pairs, and then must slowly "reset" itself in order to continue its unwinding cycle.

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Effects of Hfq on the Conformation and Compaction of DNA

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Hfq is a bacterial pleiotropic regulator that mediates several aspects of nucleic acids metabolism. The protein notably influences translation and turnover of cellular RNAs. Although most previous contributions concentrated on Hfq's interaction with RNA, its association to DNA has also been observed in vitro and in vivo. Here, we focus on DNA-compacting properties of the Hfq hexamer as well as the NTR and CTR regions. Various experimental technologies, including fluorescence microscopy imaging of single DNA molecules confined inside nanofluidic channels, atomic force microscopy and small angle neutron scattering have been used to follow the assembly of Hfq on DNA. Our results show that Hfq forms a nucleoprotein complex, changes the mechanical properties of the double helix and compacts DNA into a condensed form. We propose a compaction mechanism based on protein-mediated bridging of DNA segments. The propensity for bridging is related to multi-arm functionality of the Hfq hexamer, resulting from binding of the C-terminal domains to the duplex.

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Effects of H2A Histone Variants on DNA Sequence and Nucleosome Structure using Coarse Grain Simulations

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Coarse grain molecular dynamics (CG-MD) simulations are seeing a rising tendency in a wide range of applications because their potential to enhance the sampling of all-atomistic simulations (AA-MD) and the capacity to make near-atomistic millisecond-timescale simulations practical, putting the second threshold on the horizon. In this field, the coarse-grained Martini force field has a prominent position and has recently extended its already range of applications to DNA-containing biomolecules [1].

Eukaryotic genomic DNA exists as highly compacted nucleosome arrays called chromatin. Each nucleosome contains a 147-base-pair (bp) stretch of DNA, which is sharply bent and tightly wrapped around a histone protein octamer [2]. Several mechanisms regulate DNA accessibility, including replacement of canonical histones with specialized histone variants. Among the core histone variants, the H2A family is the biggest one -to date 5 histones variants are documented- showing also the highest sequence divergence among histone families. H2A variants show striking differences in three sites that are critical in intra- and inter-nucleosome interactions: the docking domain, which is close to the DNA entry/exit region, the L1 loop and the acidic patch, which is involved in nucleosome-nucleosome interactions. We have studied the structural and dynamic differences in those regions among H2A histone variants-containing nucleosome structures. Furthermore, it has been shown that DNA sequence effects vary between nucleosomes resulting in a significant factor in their stability. For this reason, we have also analyzed the differences in the associated DNA flexibility along the wrapped DNA sequence derived from MD simulations.

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2771-Pos Board B148

Effector-Free Molecular Mechanism of Epigenetic Regulation Revealed by Molecular Dynamics Simulations and Single-Molecule FRET Experiments

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Although the genomic DNA of a eukaryotic cell encodes for all of its genes, what really control the cell's fate is epigenetic markers that determine activation or repression of the genes. The known epigenetic markers include methylation and acetylation of the N-terminal tail of histone proteins and methylation